

Bovine Viral Diarrhea Virus in Swine: Characteristics of Virus Recovered from Naturally and Experimentally Infected Swine

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ABSTRACT

A noncytopathogenic field strain of bovine viral diarrhea virus (BVDV) was isolated from an Iowa farm brood sow and from her hysterectomy-derived, colostrum-deprived (HDCD) piglets. This field isolant was fully virulent for a neonatal calf. The NADL strain of BVDV was passaged through a series of HDCD piglets with no resultant loss of virulence for neonatal calves. Most of the BVD viral isolants recovered from pigs had been changed from a cytopathogenic biotype to a noncytopathogenic biotype. Circumstantial evidence points to swine as "carrier" hosts of BVDV.

RÉSUMÉ

On a isolé une souche non-cytopathogène du virus de la diarrhée à virus bovine (BVDV) chez une truie provenant d'une ferme de l'Iowa, ainsi que chez ses porcelets qu'on avait obtenus par hystérectomie et privés de colostrum (HDCD). Cette souche s'avéra tout à fait pathogène pour un veau naissant. On effectua des passages de la souche du virus de la diarrhée à virus bovine du Laboratoire National des Maladies Animales, chez plusieurs porcelets HDCD, sans en amoindrir la virulence pour les veaux naissants. La plupart des souches du virus de la diarrhée à virus bovine isolées chez des porcs avaient perdu leurs propriétés cytopathogènes. Une évidence circonstancielle laisse supposer que le porc agit comme "porteur" du virus de la diarrhée à virus bovine.

INTRODUCTION

Immunological relationships between bovine viral diarrhea (BVD) and hog cholera (HC) viruses have long been known (6, 7, 15, 17, 21, 23) and have prompted different groups of investigators to attempt immunization of swine with live BVD viruses (1, 3, 4, 20, 27). Baker *et al* (3) concluded that live BVD viral vaccine was safe and could not spread from vaccinated pigs to other pigs, nor to susceptible cattle kept in close contact with them. Tamoglia *et al* (27) demonstrated that, although BVD viral vaccines had some value in protecting pigs against challenge with field isolants of HC viruses, they would not protect as well as modified live virus HC vaccine, nor would they meet the U.S. Department of Agriculture testing requirements. Beckenhauer *et al* (4) speculated that even though they did not succeed in infecting control pigs by contact with pigs inoculated with BVD virus, it is entirely possible that BVD virus could be successfully passed through swine and back into cattle; this phenomenon would parallel the situation where HC virus can be passed numerous times through rabbits and is still capable of changing back into virulent HC if it is serially passed from pig to pig (4). Snowden and French (25) suggested that the presence of BVD viral antibodies in pigs could indicate that BVD virus may be infecting pigs in Australia, even though no one has reported recovering BVD virus from pigs under natural conditions. Carbrej *et al* (5) exposed 40 pigs to the NADL strain of BVD virus and determined that two pigs were positive for BVD by the fluorescent antibody cell culture technique (FACCT) test; however, the presence of BVD virus was confirmed by calf inoculation of splenic ma-

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terial for only one of the two FACCT-positive pigs. In a later study, Stewart *et al* (26) exposed 66 pigs to either the NADL strain, the Singer strain of BVD virus, or commercial BVD vaccines. Evidence of viremia was established by the isolation of BVD virus (FACCT) from seven pigs: four of ten pigs given the Singer strain, three of 46 pigs given the NADL strain, and none of ten pigs given commercial BVD vaccines. Neutralizing antibody titers against BVD virus developed in nine pigs given the Singer virus; all other pigs in these experiments were sacrificed in attempts to recover viral agent by FACCT from their various tissues. The only clinical reaction of 46 pigs exposed to NADL virus was a febrile reaction which reached 105°F in some swine. A group of ten pigs penned with three BVD virus-infected calves responded only with elevated body temperatures, and BVD virus was not recovered from any of these pigs, thus BVD viral infection could not be confirmed.

The first paper (9) in the present series reports the occurrence of BVD virus-neutralizing antibody in naturally and experimentally infected swine. The present paper reports attempts to isolate BVD virus from naturally and experimentally infected pigs, and describes the characteristics of these isolants.

MATERIALS AND METHODS

EXPERIMENTAL SWINE

Brood sows from Iowa farms, their hysterectomy-derived, colostrum-deprived (HDCD) pigs and naturally-farrowed, specific pathogen free pigs from the National Animal Disease Laboratory closed herd (NADL-SPF pigs) utilized in this study have been described (9).

EXPERIMENTAL CALVES

Two neonatal colostrum-deprived Holstein-Friesian bull calves (nos. 6570 and 6553) from a local dairy herd were given orally 10 ml of a third EBK cell culture-passaged NADL strain of BVD virus (NADL-EBK_s) containing approximately 10^{6.5} cell culture infective doses, 50% (CCID₅₀) per ml. These calves were held

in isolation as controls on pig-isolated and pig-passaged BVD viruses.

A "caught" colostrum-deprived calf (no. 7027) from the NADL BVD-negative SPF herd was used to establish the calf-infectivity of a BVD viral isolant from naturally-infected farm pigs. When approximately two hours old, the calf was given orally 25 ml of a pool of fifth through eighth bovine turbinate (BT) cell culture-passaged viral isolant derived from a HDCD pig (no. 6561). The viral titer of this inoculum was approximately 10^{7.0} CCID₅₀ in BT cell cultures.

Neonatal (one to two days old) colostrum-deprived Holstein-Friesian bull calves procured from a local dairy herd were used to test the pathogenicity of pig-passaged BVD viruses. Specific protocols are outlined in a section to follow.

INDEX OF ILLNESS OF CALVES

A numerical index of illness was developed to compare and evaluate inactivated BVD viral vaccines (10, 11, 19). This index can be useful, also, to compare the relative virulence of different strains or isolants of BVD virus. Earlier applications of this index of illness did not place proper emphasis on calves that died from acute BVD disease; therefore, in the present study, a score of 100 points minus the number of days calves lived after infection with BVD virus, was given to calves that died. This weighted value compensates for the absence of points assigned for clinical signs when calves infected with BVD virus die very early in the course of the disease (18). This modified index of illness was applied to calves infected with pig-passaged, and cattle-isolated BVD viruses and with a field isolant from pigs in order to determine any differences in virulence of the various isolants.

VIRUS

Low passage levels of the NADL strain¹ of BVD virus in embryonic bovine kidney (EBK) cells were employed as pig inocula, and as neutralizing virus or challenge virus in neutralization tests.

A BVD virus isolated from an outbreak of disease in vaccinated cattle in St. Anthony, Iowa, and characterized as being

¹Available from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852, U.S.A. (ATCC No. VR-534).

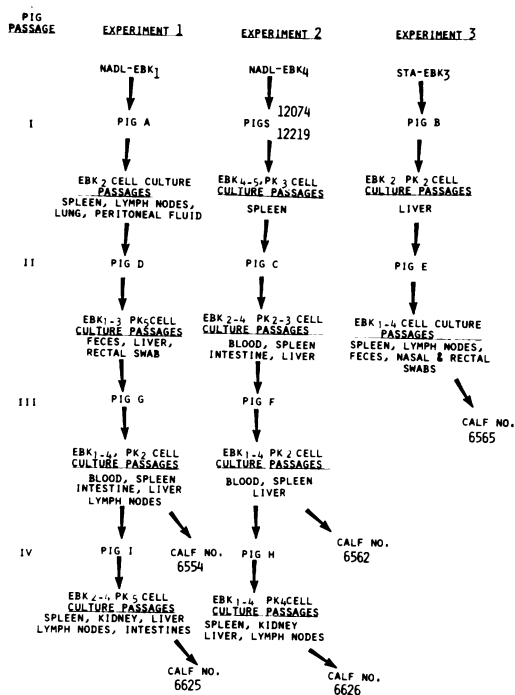


Fig. 1. Flow chart showing oral passage of BVD viruses in pigs and alternate cell culture passages.

closely related to the C24V strain biotype and serotype (12) was used in a pig passage experiment. This isolant has been designated the StA strain and biotype CP-V as contrasted to the NADL strain as biotype CP and noncytopathogenic strains as biotype NCP.

NEUTRALIZATION TESTS

Neutralizing serotiters of naturally infected swine or of swine and calves experimentally infected with BVD virus were determined as previously reported (9).

IDENTIFICATION OF BVD VIRUS FROM SWINE

The BVD viral agents recovered from field cases or experimentally infected swine were identified by immunofluorescence, by serum neutralization tests as described earlier (8, 12), and by production of BVD in susceptible calves.

PASSAGE OF BVD THROUGH SWINE

A scheme of alternate passages of BVD viruses in pigs and in susceptible cell cultures similar to that earlier described for

rabbit adaptation (14) was employed. Biotypes of viruses isolated from pigs were determined by a previously reported method (12). Pig tissues inoculated into EBK or PK-15 cells were considered to be negative for BVD virus after four passages if neither CPE nor interference of CPE (8) was shown to be present.

Experiment 1 — Fifteen ml of a first EBK cell culture passage of the NADL strain of BVD virus (NADL-EBK₁) containing approximately $10^{6.5}$ CCID₅₀ per ml was given orally to a three day old HDCD pig (pig A, Fig. 1). Virus isolated from the tissues of this pig by cell culture was given orally to pig D, a 24 day old HDCD pig, then virus cultured from tissues of pig D was given orally to an HDCD one day old pig (G). Virus from pig G was then fed to a 33 day old NADL-SPF pig (I) as outlined in Fig. 1. Cyclic passages of virus from cell culture to pig are indicated by Roman numerals appearing to the left of the pig designation. Pooling of inocula are indicated under the line designating cell cultures which were positive for BVD virus. The subscript numbers (e.g. EBK₄) indicate the passage levels of cells supporting BVD virus isolated from the indicated tissues. Cell cultures of pooled viruses isolated from pigs were given orally in 15 ml amounts to calves 6554 and 6625 (Fig. 1 and Table I). Severity of illness of calves was expressed as an index of illness value.

Experiment 2 — The NADL strain of BVD virus passaged four times in EBK cells (NADL-EBK₄), then passaged through two NADL-SPF pigs 60 to 90 days old (nos. 12074 and 12219) was obtained from Dr. E. A. Carbreys² as a spleen suspension (5). After several passages in EBK and PK-15 cells, 15 ml of a cell culture suspension of this virus containing approximately $10^{6.3}$ CCID₅₀ per ml was inoculated orally into a three day old pig (pig C, Fig. 1). Cyclic passages were made from cells to pigs F and H (one day old HDCD and 33 day old NADL-SPF animals, respectively). Fifteen ml of pooled virus from pigs F and H were given orally to calves 6562 and 6626 (Fig. 1 and Table I).

Experiment 3 — Fourteen ml of a third EBK-passaged StA strain of BVD virus

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TABLE I. Results of Passage of BVD Viruses in Neonatal HDCD Pigs

Experiment No.	Pigs			Pig Passage		Virus Isolation		Cells and Passage No.	Viral Titer ^a (Log ₁₀)	Biotype of Isolants	Calf Infectivity
	No.	Source	Age (Days)	No.	Days	PI	Source				
1	A	HDCD	3	1	7-D ^b 21		Spleen, lymph nodes, peritoneal fluid	EBK ₂	6.3	CP	ND ^c
	D	HDCD	24	2			Feces	EBK ₂	6.0	CP	
	G	HDCD	1	3	8		Rectal swab	EBK ₃	6.3	NCP	ND
							Liver	PK ₅	6.5	NCP	
2	F	HDCD	1	3	8		Blood, spleen, liver, lymph nodes, intestines	EBK ₄	6.5	NCP	Infected calf no. 6554
							Liver	PK ₂	7.0	CP	
	H	SPF	33	4	20		Spleen, kidney, liver, lymph nodes, intestines	EKB ₄	6.5	NCP	Infected calf no. 6625
							Kidney, liver	FK ₅	7.0	CP	
3	12074	SPF	60-90	1	3		Spleen	PK ₃	6.0	NCP	ND
	12219	HDCD	60-90	2	7		Spleen	EBK ₅	6.5	NCP	
	C	HDCD	3	2	17-D		Blood, spleen, intestines	EKB ₄	6.5	NCP	ND
							Liver	PK ₃	6.0	NCP	
2	F	HDCD	1	3	8		Blood, spleen, liver	EBK ₄	6.3	NCP	Infected calf no. 6562
							Liver	PK ₂	6.8	CP	
	H	SPF	33	4	20		Spleen, kidney, liver	EBK ₄	6.0	NCP	Infected calf no. 6226
							Lymph nodes	PK ₄	5.5	NCP	
3	B	HDCD	3	1	22		Liver	EBK ₂	6.5	NCP	ND
	E	HDCD	24	2	21		Liver	PK ₂	7.0	CP	
							Spleen	EBK ₂	6.5	CP-V	Infected calf no. 6565
							Lymph nodes, feces	EBK ₂	6.0	CP	
3	E	HDCD	24	2	21		Rectal swab	EBK ₂	6.5	CP	
							Nasal swab	EBK ₃	6.2	NCP	
	E	HDCD	24	2	21		Liver	PK ₅	6.5	CP	

^aRefers to the CCID₅₀ per ml of pooled viruses from the indicated tissues passaged in the indicated cell cultures. ^bD = pigs died. ^cND = not done.

containing approximately $10^{6.5}$ CCID₅₀ per ml was given orally to a three day old HD CD pig (B), then a pool of viruses isolated in EBK and PK cells given orally to a 24 day old HD CD pig (E). Viruses isolated by cell culture passages were verified as BVD viruses in calves (Fig. 1), and their relative virulence determined by an index of illness.

RESULTS

ISOLATION OF BVD VIRUS FROM A BROOD SOW AND HER HD CD PIGS FROM AN IOWA FARM.

Noncytopathogenic BVD viral agents were isolated from the leukocytes of one sow and three of her HD CD pigs (Table II).

TABLE II. Isolation of a Field Strain of BVD Virus from a Brood Sow and Her HD CD Pigs and the Development of Neutralizing Serotiters over a Ten Week Period

Pig No.	Testing Period (Weeks)					
	Zero		Seven		Ten	
	Virus Isolation	Neutralizing Serotiter	Virus Isolation	Neutralizing Serotiter	Virus Isolation	Neutralizing Serotiter
27 (Sow) . . .	+	16	NT ^a	NT ^a		
6560	—	16	—	64	—	64
6561	+	0	+	0	—	0
6562	—	0	—	0	—	0
6563	—	32	—	512	—	256
6564	—	0	NT ^b	NT ^b		
6565	+	0	+	0	+	0
6566	—	0	—	0	NT ^b	NT ^b
6567	—	0	+	0	NT ^b	NT ^b
6568	—	0	NT ^b	NT ^b		
6569	—	0	NT ^b	NT ^b		

^aNT = not tested; sow killed at hysterectomy
^bNT = not tested; pig died

TABLE III. Index of Illness Evaluation of Calves Inoculated with a Pig Is olant, with Pig-Passaged BVD Viruses and with the NADL Strain of BVD Virus

Persistence of clinical signs (Days)												
BVD Virus Given	Calf No.	Virus Given	Rectal Temp. Elevation	Leukopenia	Enteritis	Respiratory Distress	Depression	Anorexia	Lameness	Recovery of Virus (Days)	Death of Calf	Index of Illness
NADL strain from cattle	6570	NADL-EBK ₃	3	3	6	3	2	2	0	13	0	32
	6553	NADL-EBK ₃	3	0	3	3	2	2	2	3	94	112
Field isolant from pigs	7027	pig 6561	2	0	8	0	2	2	8	9	88	119
Experimentally pig-passaged	Exp. 1	6554 Pig G	34	8	10	5	12	6	0	30	0	105
		6625 Pig I	6	5	3	4	6	5	0	0	77	106
	Exp. 2	6562 Pig F	15	5	4	0	3	2	10	8	0	47
		6226 Pig H	17	6	11	5	5	6	0	42	0	92
	Exp. 3	6565 Pig E	5	5	5	3	5	5	4	7	93	132

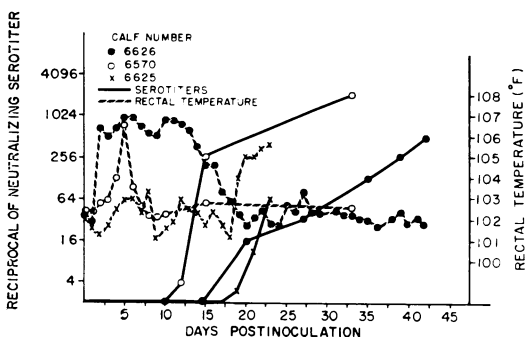


Fig. 2. Neutralizing serotiters against the NADL strain of BVD virus and rectal temperatures of two colostrum-deprived dairy farm calves given pig-passaged BVD virus and one calf given the NADL strain of BVD virus.

This sow was located on a farm (MB) where antibodies against BVD virus had previously been detected in several sows and HDCD pigs, and where swine were kept in close proximity to cattle (9). Although BVD virus was isolated from this sow, she had a neutralizing serotiter of 16 against BVD virus; two piglets, other than those from which BVD virus was isolated, had initial neutralizing serotiters of 16 and 32. The BVD virus was isolated from pig 6561 at zero and seven weeks after hysterectomy, from pig 6565 at zero, seven and ten weeks and from pig 6567 at seven weeks post-hysterectomy. The identity of the noncytopathogenic viral isolants was established by immunofluorescence and by neutralizations with specific anti-BVD sera.

Further confirmation that these pig-isolated viruses were BVD viruses was obtained by the production in an SPF calf of severe BVD which terminated in death (Table III).

PRODUCTION OF BVD IN CALVES INOCULATED WITH THE CATTLE-ISOLATED NADL STRAIN OF BVD VIRUS

The low-passaged NADL strain of BVD virus produced a fairly mild disease in control calf No. 6570, and fatal BVD in control calf No. 6553 (Table III). Virus was re-isolated from calf No. 6570 and a serotiter of 2048 developed 33 days postinfection (Fig. 2). Virus was recovered from nasal swabs and feces of calf No. 6553 three days postinfection, and the calf died on the sixth day; therefore, no neutralizing antibodies were produced.

EXPERIMENTAL INFECTION OF HDCD PIGS WITH BVD VIRUS

Experiment 1 — The NADL strain of BVD virus was successfully passaged with alternate cell culture passages through three HDCD and one NADL-SPF pig (Fig. 1 and Table I). The biotype of re-isolated viral agents consisted of either CP or NCP isolants with no apparent consistency as to biotype isolated from a particular cell culture (Table I, Column 10). Virus titers ranged from 5.5 to 7.0 (\log_{10}) CCID₅₀ per ml (Table I, column 9) in the cell line and passage number indicated (Table I, column 8). The identity of the viral agents isolated from pigs was confirmed by neutralization and immunofluorescence with specific anti-BVD serum. Further confirmation of the identity of viruses isolated from pigs G and I was the production of severe BVD in calves No. 6554 and No. 6625 expressed as an index of illness (Table III). Plots of rectal temperatures and development of neutralizing serotiters in calf No. 6625 are shown in Fig. 2. From these data one can conclude that calves inoculated with pig-passaged BVD virus developed severe and clear-cut cases of BVD.

Experiment 2 — Bovine viral diarrhea virus was recovered from several tissues and organs of the five pigs given BVD virus originating from the NADL strain. Again, the identity of viral agents isolated from the pigs was established as BVD virus by CPE or interference with CPE and by neutralizations and immunofluorescence with specific anti-BVD sera. Contrasted with experiment 1, more isolants consisted of biotype NCP than biotype CP (Table I, column 10). Viral isolants from pigs F and H produced typical and severe BVD in calves Nos. 6562 and 6226 (Table III).

Further confirmation that the infecting virus was a BVD virus was the development of a virus-neutralizing serotiter in calf No. 6626 (Fig. 2).

Experiment 3 — The StA strain of BVD virus, originally classified as biotype CP-V, produced severe BVD in calf No. 6565 after two cyclic pig-cell culture passages (Tables I and III). Only the isolants from spleen, lymph nodes and feces of pig E cultured on EBK cells retained the CP-V biotypic

characteristic; liver homogenates from both pigs B and E cultured in PK-15 cells contained the CP biotype; virus from all other tissues cultured on EBK cells was of the NCP biotype (Table I, columns 7 and 10).

DISCUSSION

Baker *et al* (3) stated that BVD vaccine virus is safe for use in swine as it cannot be spread from vaccinated pigs to other pigs, nor spread from vaccinated pigs to susceptible cattle kept in close contact. Admittedly, we did not conduct any experiments to disprove these statements; we did, however, show that virulent strain of BVD virus will propagate in neonatal swine and in pigs as old as 30 to 90 days, and retain infectivity for periods as long as three weeks; the BVD viral isolants from these pigs were extremely virulent for neonatal calves. Furthermore, serial passage of BVD virus in swine for as many as four passages did not attenuate its virulence for cattle. Additionally, BVD virus is shed in nasal mucus and feces of infected pigs, thus making pigs prime suspects as "carriers" of BVD viruses, quite the opposite of the dead-end hosts suggested by Snowden and French (25).

Isolation of a field strain of BVD virus from a naturally infected sow and her piglets provided additional proof that swine can harbor BVD viruses. This field isolant was as virulent or perhaps more virulent for neonatal cattle than the NADL strain of BVD virus. Although the evidence is circumstantial that swine are infected with BVD virus by co-mingling with infected cattle, isolation of a field strain of BVD virus provides convincing evidence of its authenticity. Cross-infectivity experiments between cattle and swine are currently being conducted in our laboratory to further elucidate the role of swine in the cattle disease BVD.

We certainly could not recommend the use of live BVD viral vaccines in swine as has been recommended by several workers as a control measure against HC (1, 2, 3, 4, 20, 22, 28), but would join with those who believe that such practices either may not be effective or may result in harmful consequences (17, 24, 26, 27). Furthermore, officials in the U.S. Department of

Agriculture have issued a Biological Products Notice against the use of BVD vaccines in swine (16).

Of particular interest is the re-isolation of BVD viruses from swine and the accompanying change of biotypes, a phenomenon which has been encountered with BVD virus propagated in certain cell line cultures (13) and in rabbits (14). These biotypic changes were not too surprising, since it was earlier proposed that an intermediate host such as the swine may be responsible for the occurrence of variants or strains of BVD virus in nature (13). Possible changes in serotypes with the changes in biotypes (12) in the present study have not yet been determined for swine-passaged variants of BVD virus.

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ERRATA

Can. J. comp. Med. 36: 393-397. 1972.

The first sentence of the Abstract of this article should read "Forty-eight intact male pigs were used to investigate the influence of source of protein supplement, corn moisture content, and supplemental vitamin E and selenium on the incidence of mulberry heart disease, hepatosis dietetica and associated lesions."

The editors regret any inconvenience caused.